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RAPID ESTIMATION OF POST EXPOSURE INCAPACITATION: 1. DEFINITION OF THE ROTOPAD AND SPASM TESTS (U)

by

R.W. Bide and D.J. Risk

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DRES Animal Care Statement

In conducting the research described in this report, the investigators adhered to the "Guide to the Care and Use of Experimental Animals" published by the Canadian Council on Animal Care.

ABSTRACT

A simple test to indicate impaired motor function in mice is described. The test can be completed within 2 min using naive mice. Training of the animals is not a requisite. In normal mice, 94.7% of the population pass the test. Impaired motor function (Significant $P < 0.01$) is indicated when 2 of 5 animals fail the test. The effects of sodium pentobarbital, diazepam and chlorpromazine were studied to validate the procedure. All three drugs produced impaired function. Halothane anesthesia (by inhalation exposure) was also tested but the recovery of the mice was too fast for reliable estimates of impaired motor function to be obtained.

A second test is described in which the tonic convulsions that occur spontaneously in CD-1 mice when they are suspended by the tail are used as a measure of seizure activity. Enhancement and suppression of the convulsions can be demonstrated. Chlorpromazine, halothane, diazepam and sodium pentobarbital all suppressed the spasm, the latter two at doses much below those required for effect in the rotopad test.

RÉSUMÉ

On décrit un test simple qui révèle les atteintes de la fonction motrice chez la souris. Le test peut être réalisé en deux minutes avec des souris naives. Il n'est pas nécessaire d'entraîner les souris. Sur une population de 34 souris normales, 94,7% ont réussi le test. On conclut à une atteinte de la fonction motrice ($P < 0,01$) lorsque 2 animaux sur 5 ne réussissent pas le test. On a étudié les effets du pentobarbital sodique, du diazépam et de la chlorpromazine pour valider la méthode. Ces trois médicaments ont amené une altération de la fonction motrice. On a également testé l'anesthésique halothane motrice. On a également testé l'anesthésique halothane (exposition par inhalation), mais les souris se sont rétablies trop rapidement pour que l'on puisse obtenir des données fiables sur l'atteinte de la fonction motrice.

On décrit un autre test dans lequel on utilise comme mesure de l'activité durant un épisode de crise les convulsions toniques qui surviennent spontanément chez la souris CD-1 lorsqu'elle est suspendue par la queue. On a démontré la possibilité d'accroî tre ou de supprimer les convulsions. La chlorpromazine, l'halothane, le diazépam et le pentobarbital sodique ont éliminé les spasmes; les deux derniers produits ont été administrés à des doses nettement inférieures à celles qui ont été nécessaires pour produire un effet au cours du test «rotopad».

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INTRODUCTION

Non-lethal effects caused by chemical exposure can result in many casualties or the loss of military function/effectiveness in exposed troops. These effects include loss of coordination, mental derangement, blinding and respiratory distress and incapacitation. The chemical effect can be transient as with an anaesthetic or can be a permanent chemical burn as caused by mustard. It follows that assessments of the military importance of potential warfare chemicals should include a measure of the incapacitation to be expected during and following chemical exposure.

The assessment, in laboratory animals, of impaired motor function immediately following exposure to noxious chemical atmospheres, requires a simple, rapid test. There should be little or no manipulation of the animals so that tension, excitement and manipulative interference are minimized. The test should have a definite end or test point that is neither subjective nor requiring of interpretation to eliminate variability caused by different operators. Further, if the test can be done with naive animals the man-hours required for animal training can be avoided.

The ability of mice to balance upon a rotating rod is often used as a measure of motor function (1, 2, 3, 4, 5), but the animals must first be "trained" in the procedure. A simpler test of muscle coordination and grip strength, first proposed by Kondziella (6), placed mice on an inverted wire grid from which impaired animals would fall within a specified period. This concept was refined by Coughenour, McLean and Parker (7), who placed the mice on top of a wire mesh pad, inverted the pad and recorded the proportion of animals that failed to climb to the top of the pad and the proportion of animals that fell from the grid. For this "Rotopad" test, a combination of mental function, motor coordination and grip strength were required for successful completion of the test, *ie.* climbing to the top of the pad within 60 sec. Variations of this Rotopad test, with both naive and trained animals, have been used to assess motor function following a number of treatments and drug regimens (8, 9, 10, 11, 12).

During the set-up and validation of the "Rotopad" test described above, it was noted that a proportion of the animals being transferred to the pads showed tonic convulsions (spasm) and, in animals that did not respond initially, gentle rotation of some animals when suspended by the tail induced a similar spasm response. The incidence of spasm was also recorded during the validation trials. This report describes the work done to validate the Rotopad and Spasm tests. The effects of body weight, sex and various drug treatments were explored to define and refine the range and applicability of the tests.

METHODS

The mice used in these tests were of the CD-1 outbred albino strain purchased from Charles River Laboratories, St. Constant, Que. The animals were acclimatized in the Vivarium at the Defence Research Establishment Suffield for at least 7 days before use. Before each test, each animal was weighed, marked for identification with a non-toxic felt pen (VWR Lab Markers, permanent alcohol/waterproof, black; VWR Scientific, San Francisco, CA.) and returned to the original cage.

Drugs used were Largactil 50 (chlorpromazine hydrochloride, 27.9 mg/ML in isotonic saline; DIN 163953; Rhone-Poulenc Inc. Montreal), diazepam (5 mg/ML in propylene glycol:ethanol:H_2O (4:1:5) with 4.25% benzoic acid and 1.5% benzyl alcohol; DIN 399728; SABEX, Boucherville, Quebec) and sodium pentobarbital (65 mg/mL solution in aqueous propylene glycol base; DIN 141690; M.T.C. Pharmaceuticals, Cambridge, Ontario). Halothane, 2-bromo-2-chloro-1,1,1-trifluoroethane (DIN 346314), was obtained from MTC Pharmaceuticals, Hamilton, Ont.

A Rotopad apparatus was constructed from a 5 cm pipe suspended by brackets from bearings and turned by handles at the ends (Fig. 1). Eleven, 12 x 12 cm "pads" of 2 mm stainless steel wire mesh were mounted on 6 mm rods and fixed in threaded holes at equal intervals in the pipe. The height of the pads above the rod was set so that the pads were in line with the suspending bearings to produce an minimum displacement of the pads when the rod was turned. The object was to be able to invert the "pads" by

rotating the rod to induce the mice to climb to the top of the pads (Fig. 2). A simple latch was included to hold the device in the inverted position.

For the Rotopad test, naive mice of known sex and body weight, were placed on top of the pads. At time 0, the pads were quickly inverted, in no more than 1 sec. The records included the numbers of mice that fell from the pads, the number that failed to climb to the top of the pads and, for the control, untreated mice only, the time each animal climbed to the top of the pad. If a mouse fell, it failed the test so that the failure rate is the sum of those falling and those still hanging to the underside of the pads after 60 sec.

To test for the tonic convulsion (spasm) response, the mice were picked up by the distal portion of the tail and held head down for 5 sec. If the spasm response (Fig. 3) was observed, the mouse was counted as showing spontaneous or direct spasm. If there was no spasm, the animal was rotated and then allowed to hang for 5 sec. If there was no spasm this was repeated once. Mice showing spasm following rotation were scored as showing induced spasm.

Test scores for the Rotopad and the spasm tests were compiled and compared as incidence values using the Chi-square test of proportions (13). Data for naive untreated animals of both sexes were accumulated to provide a data base for test comparisons.

All doses of drugs were administered *i.p.* The drugs were diluted in isotonic saline such that for each dose the mice were injected with a constant volume per body weight. With all drugs, the doses were given at time 0 and the mice were tested on the Rotopad and for spasm at 30, 60, 90 and 120 min post injection. These times were chosen to coincide with the original method report of Coughenour *et. al.* (7).

The gaseous anaesthetic, halothane, was delivered by inhalation using a recirculating exposure system which included a MIRAN infra red analyzer to follow the concentration of substance in the exposure gas. The exposure system was calibrated daily by serial injections of halothane.

RESULTS

The population of normal, naive animals

Rotopad test

Five hundred and thirty one naive mice consisting of 202 females and 329 males were tested on the Rotopad to define the normal population (Table I). Slightly more than 1% of the mice fell from the pad and 5.3% failed the test. The proportions of males and females that failed the test, 5.5% and 4.5% respectively, were not significantly different (Table II). The proportions that fell from the pads, 1.5% and 0.5% respectively, were also statistically similar. When the data were assembled into groups for each sex by body weight using 5 g intervals, there were no significant differences (Chi-square; $P > 0.05$) between groups either within or between the sexes.

The times required for the mice to climb to the top of the pad were grouped and the time distributions plotted (Fig. 4). The females had a bimodal population with modes at 20 - 30 sec and 40 - 50 sec (The 30 - 40 sec group was significantly different ($P < 0.01$) from the groups on either side). The males had a broader distribution with the mode in the 20 - 30 sec group. In each case, <2% of the population was found in the 50 - 60 sec groups.

In an attempt to explain the distribution found in the female mice, the data was further separated into groups by body weight in ranges of 5 g. Of the mice which responded in the 40 - 50 sec time group, the majority (17 of 23 mice) were found in the 25 - 30 g weight group (Table III). Of the 5 mice in the 20 - 25 g weight group, 3 of 5 were found in the 40 - 50 sec group. When the 25 - 30 g weight group was further separated into 1 g groups, the mice responding in the 40 - 50 sec were found to be evenly distributed by weight and in proportion to those responding in 20 - 30 sec. Therefore, body weight and, by inference, age were not the cause of the bimodal distribution observed in the female mice.

Spasm test

Four hundred and sixty nine mice, 173 females and 296 males, were tested for spasm. None of the mice exhibited direct spasm. Induced spasm was recorded in about 20% of the male population without regard to age and/or size/weight (Table I, II). In the female population the incidence of spasm was significantly higher ($P<0.01$) and there was a significant increase in the incidence of spasm with increasing age/weight. The largest/oldest females, weighing >35 g, showed an 80% incidence, albeit that the sample size was small (N = 16), and the smaller/younger 25 - 30 g weight group had an incidence of 28.6%.

Effects of drugs

Sodium pentobarbital

With sodium pentobarbital at time 30, the ED_{50} (Effective Dose for 50% incidence of effect) was 26 (21.3 : 29.7) mg/kg[1] in the Rotopad test (Table IV). The ED_{50} values for 60, 90 and 120 min were higher (30.8 mg/kg) but the potency ratio was significantly different ($P < 0.01$) only at 60 min because of the increased variation in the 90 and 120 min data. All of the affected mice fell from the pads. There was no transition from falling to test failure; none of the animals who failed the test remained upon the grid. In animals given sodium pentobarbital the spasm response was completely suppressed by doses of sodium pentobarbital ≥0.5 mg/kg. At 0.1 mg/kg, 1 of 5 mice showed induced spasm.

Diazepam

With diazepam, the ED_{50} for animals that fell from the pads at time 30 was 0.58 (0.25 : 1.54) mg/kg in the Rotopad test. The ED_{50} values increased steadily for 60, 90 and 120 min so that the potency ratio was significantly reduced for 120 min value of 2.88

[1] Values for ED_{50} are given as the ED_{50} (95% confidence limits). Calculations were done using a computer program based upon the method of Litchfield and Wilcoxin (@13).

mg/kg. At 30 min, there were two mice that failed the test but did not fall. When these were accounted for in the calculation the ED_{50} value was reduced to 0.48 mg/kg. At 60, 90 and 120 min, more mice failed the test but did not fall and the ED_{50} values for affected animals reflect this change (Table IV). None of the mice that were given doses of diazepam ≥0.2 mg/kg, showed any signs of direct or induced spasm. Of the mice given 0.02 mg/kg diazepam, 2 of 5 showed induced spasm at all test times.

Chlorpromazine

Chlorpromazine caused impaired motor function over a wide range of doses but unlike the drugs above, all of the animals in each group were not affected. Between 3 and 15 mg/kg, 1 of 5 mice in each dose group or 5 of 30 mice (Table V) were not affected at time 30 min. At 60 and 90 min, the effect of chlorpromazine increased so that 29 of 30 mice in this dose range were affected and fell from the pads. At 120 min, the mice were beginning to recover from the drug and again some mice in this dose range failed the test but did not fall. Between doses of 0.5 and 1.5 mg/kg (0.5 and 3 mg/kg at 120 min), there was a transition from all mice falling from the pads to test failure with the mice remaining suspended under the pads. At 120 min, The potency ratios between 30 and 120 min were significantly different for both mice that fell and mice that failed the test. With doses ≤0.1 mg/kg there was no apparent effect in the Rotopad test. None of the mice given >0.1 mg/kg showed any sign of spasm. Of the mice given 0.1 mg/kg, 4 of 5 showed induced spasm at 30 and 60 min (Significant increase compared to control population; $P < 0.01$) and 2 of 5 showed induced spasm at 90 and 120 min.

Halothane

Halothane, delivered in the recirculating exposure system at 1%, 3% and 4% (v:v), produced a clearly visible anaesthetic effect while the exposure was in progress. During the 8 min purge of the exposure system, mice exposed to 1% halothane recovered and were apparently unaffected at 10 min post exposure when tested on the Rotopad and for

spasm. Mice exposed to 3% halothane were still significantly affected as indicated by the significant failure rate on the Rotopad test. The spasm response was completely suppressed by the exposures to halothane used in this study.

DISCUSSION

The Rotopad test described in this report appears to provide a fast and simple measure of impaired motor function and grip strength that can be applied and completed within a very short time after exposure to drugs or chemical inhalants. The test is essentially unaffected by the age and sex of the animals. It is much faster than the inclined screen test which can take 30 min to complete (14). In comparison to the more common rotating rod tests, no training of the animals is required (7) and there are fewer parameters to control (or vary) *eg.* rod size, speed and acceleration (15). Further, because there is no training requirement, there are fewer control failures and this, coupled with the lower variability, means that fewer animals are needed to achieve the same results. However, because the test uses a fixed time and because the normal incidence of failure is low, there is no opportunity to measure an increase in performance.

The test originally described by Coughenour *et.al.*(7) was not validated by trials of sex age and weight of the animals. Other authors using the test, equally did not report validation trials (8, 9, 10, 11, 12). Given the sensitivity of the test and the need to reduce the numbers of animals used, the validation was considered necessary. No significant differences were found that were related to sex or the age/weight of the mice. In the normal population of mice, the majority of the animals climbed to the top of the pad in <40 sec. About 5% failed to climb to the top in the allotted 60 sec and $<2\%$ fell from the pads. Thus, the 60 sec time limit for completion of the test appears to be justified and impaired motor function is indicated and is significantly different ($P < 0.01$) from the norm of the total population when 2 of 5 or 3 of 10 mice fail to achieve the top of the pad in 60 sec. Similarly, when 2 of 5 or 2 of 8 animals fall from the pads a significant ($P < 0.01$) decrement in grip strength is indicated as well. Using these criteria, testing of groups of

5 mice is feasible and the sensitivity of the test is essentially the same with either 5 or 10 mice per group.

Inhalation of halothane also followed a predictable pattern (16). Anaesthesia was quickly achieved in the inhalation chamber. Recovery from lower levels of exposure was rapid and apparently complete so that a 3% (v:v) exposure level was required before impaired motor function was observed 10 min after the end of the exposure period. Thus, using the Rotopad method, longer term effects are readily estimated but rapid recovery may compromise the test and results.

Militarily important motor impairment from sedative, tranquillizer and hypnotic drug responses were readily demonstrated using the Rotopad test. The results also correspond to the physiologic activity of the drugs. Sedation with loss of motor function occurred with all three drugs but anticonvulsant activity was not observed with chlorpromazine. Sodium pentobarbital is an hypnotic, sedative and an anticonvulsant. Diazepam is a tranquillizer, anticonvulsant and skeletal muscle relaxant which may cause fatigue, drowsiness and ataxia as side *effects.* Chlorpromazine is a sedative and antiemetic which causes drowsiness and dizziness as side effects. The responses to the drugs tested, including the course of events as the drugs took effect and were cleared, were essentially similar to those reported by other workers (1, 3, 7, 5) albeit that the doses, given *i.p.* in this study, were lower than those given *per os.*

The spasm test described proved to be a sensitive measure of anti-convulsion effects and has the potential to measure convulsive effects. In each case the mice should be chosen carefully to permit the maximum sensitivity for the required outcome. The males tested showed similar responses at each age/weight interval. The females showed an increase in effect with age/weight. The anti-convulsant drugs, diazepam and sodium pentobarbital, suppressed the spasm at very low doses. The appearance of direct spasm in the test animals may indicate latent or subclinical seizure or convulsant activity. However, in this study, there was no data produced that would either confirm or deny this concept.

The spasm response appears to be a central nervous system response. The tonic convulsions were suppressed by diazepam and sodium pentobarbital, which are centrally active anti-convulsant drugs, at much lower doses than those required to produce impaired motor function. In contrast, chlorpromazine did not provide a similar effect and it is not a potent anti-convulsant drug. The atypical results described here with higher doses of chlorpromazine (Table V) also agree with the previous study (7). As the doses approach the ED_{50}, the results of the rotopad tests followed the expected progressive decrease in motor effect. The suppression of the spasm response by halothane, particularly at the 1% exposure level is interesting as it indicates that the recovery from anaesthesia was not as complete as was indicated by the Rotopad test. The authors were not able to find any previous reference to anticonvulsant activity with halothane.

In the spasm test, the incidence of the induced reaction was much higher than in the Rotopad test and the responses varied with age and sex. The result of the higher incidence was that no statistical significance could be obtained with 5 mice until 4 of the 5 were affected. When the test population is increased to 10 mice, 6 mice affected of 10 is not significantly different ($P > 0.05$) from the control population. When 7 of 10 are affected, the difference is significant at $P < 0.02$ and when 8 or more are affected, the statistical probability would be $P < 0.01$. The male and female populations were not the same in response to the spasm test. First, the induced response was different for the two populations ($P < 0.01$). The males had the same incidence of induced response (≈20%) in all weight groups and the test is, therefore, equally valid for all male mice. In contrast, within the female population, the induced response increased with higher body weight/advancing age and when the test is applied to female mice, the body weight of the subjects must be carefully controlled. Although the observed variations in the females may be the result of female physiology (17) there is also the possibility that it is a true - neurologic change with advancing age as neurologic changes and physical performance decrements have been described in aging mice (18) but not, heretofore in a sex-linked fashion.

The spontaneous spasm that was observed in the early trials and which precipitated this part of the investigation, was not observed in any control animal tested. Equally, it did not occur in the drug treated animals. With this low indicated incidence of effect, the spontaneous spasm may provide a sensitive method for the assessment of this clonic nervous response. Indeed, the presence of 2 affected mice in 5 would represent a statistically significant change in the group. As none of the mice tested after drug treatment showed the spontaneous response either, there is nothing that can be said, from the results presented at this time, regarding the utility or meaning of the direct sopasm test reaction. Further efforts may illuminate this unknown quantity.

CONCLUSIONS

1. Impaired motor function may be monitored by a simple rapid test that may be performed upon naive animals within minutes of chemical exposure. The apparatus and procedure are described. The test requires no animal training and can be performed on 10 mice in under 2 min.
2. In normal mice, 94.7% of animals pass the test. In a population of 5, impaired motor function is indicated if two animals fail the test.
3. The dose responses from sodium pentobarbital, diazepam, chlorpromazine and halothane were determined to demonstrate the validity of the test.
4. Halothane effects could be measured by the rotopad test only at high exposures. Recovery from anaesthesia was very rapid and apparently complete before the test could be performed.
5. The tonic convulsive response elicited by tail suspension of normal CD-1 mice may be used as a simple rapid test for convulsant and anticonvulsant activity of drugs and treatments. The sedative, chlorpromazine, eliminated the spasm response at the same doses that affect the rotopad test. Anticonvulsive drugs, sodium pentobarbital and diazepam suppress the spasm at doses much less than those which affect the rotopad test.

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Table I

Responses in the Rotopad and Spasm Tests: Total population

Parameter	Body Weight (Mean ± St.Dev.)	Rotopad tests			Spasm tests		
		Total Animals	Total Affected	Fell	Total Animals	Induced spasm	Direct spasm
Total population							
	32.4 ±4.3	531	28	6	321	123	0
Body weight							
>40 g	40.8 ±0.7	10	0	0	9	1	0
40≥wt>35	36.9 ±1.3	165	10	3	152	37	0
35≥wt>30	32.5 ±1.6	191	13	3	160	51	0
30≥wt>25	27.7 ±1.3	144	5	0	118	32	0
25≥wt>20	23.6 ±0.9	21	0	0	5	2	0

Table II

Responses in the Rotopad and Spasm Tests: Male and female populations

Parameter	Body Weight (Mean ± St Dev)	Rotopad tests: Total Animals	Rotopad tests: Total Affected	Rotopad tests: Fell	Spasm tests: Total Animals	Spasm tests: Induced spasm	Spasm tests: Direct spasm
Total population: males and females							
	32.4 ± 4.3	531	28	6	469	140[a]	0
Males							
Total males							
	34.4 ± 3.5	329	19	5	296	63[a]	0
>40 g	40.8 ± 0.7	10	0	0	9	1	0
40≥wt>35	37.0 ± 1.3	148	10	3	142	29[c]	0
35≥wt>30	33.0 ± 1.4	132	9	2	120	29[d]	0
30≥wt>25	28.0 ± 1.4	37	0	0	25	4	0
25≥wt>20		2	0	0			
Females							
Total females							
	29.1 ± 3.4	202	9	1	173	77[a]	0
>35 g	36.4 ± 1.5	17	0	0	16	14[b,c]	0
35≥wt>30	31.5 ± 1.4	59	4	1	54	32[b,d]	0
30≥wt>25	27.7 ± 1.3	107	5	0	98	29[b]	0
25≥wt>20	23.6 ± 0.9	19	0	0	5	2	0

[a,b,c,d] Significant difference between marked groups; Chi-square; $P > 0.01$.

Table III

Distribution of time for response of female mice in the Rotopad test

Weight Weight range	Time to climb to top of pad (sec) 0-10	10-20	20-30	30-40	40-50	50-60	>60
20≤wt<25	0	0	2	0	3	0	0
25≤wt<30	0	0	67	2	17	1	4
30≤wt<35	8	2	22	1	3	1	3
35≤wt<40	0	10	0	0	0	0	0
25	1	0	4	0	1	0	0
26	0	0	13	0	3	1	0
27	0	0	18	0	3	0	1
28	0	0	20	1	6	0	0
29	0	0	8	1	3	0	2
30	0	0	8	0	2	0	1

Table IV

Responses to drugs in the Rotopad test

Test	ED_{50} at time 30	60	90	120
Sodium pentobarbital				
Fall	26.0	30.8[a]	30.6	30.6
Affected/not up	26.0	30.8[a]	30.6	30.6
Diazepam				
Fall	0.58	1.28	1.62	2.88[a]
Affected/not up	0.48	0.79	0.83	1.53[a]
Chlorpromazine				
Fall	0.52	0.82	1.31	2.41[a]
Affected/not up	0.59	0.60	1.31	2.12[a]

[a] Significant difference in potency ratio from 30 min value (Chi-square; $P < 0.01$).

Table V

Rotopad and Spasm tests following doses of chlorpromazine

Parameter	Body Weight (Mean ± St.Dev.)	Rotopad tests: Total Animals	Rotopad tests: Total Affected	Rotopad tests: Fell	Spasm tests: Total Animals	Spasm tests: Induced spasm	Spasm tests: Direct spasm
Total population: males and females							
	32.4 ± 4.3	531	28	6	469	140[a]	0
Chlorpromazine dose							
11.84	38.9 ± 3.0	5	4[a]	3[a]	5	0[a]	0
10.0	40.5 ± 2.6	5	5[a]	5[a]	5	0[a]	0
5.0	39.0 ± 3.0	5	4[a]	4[a]	5	0[a]	0
3.0	38.8 ± 2.6	5	5[a]	5[a]	5	0[a]	0
1.5	37.4 ± 2.6	5	5[a]	4[a]	5	0[a]	0
1.0	38.2 ± 2.6	5	4[a]	2[a]	5	0[a]	0
0.5	39.1 ± 1.7	5	3[a]	3[a]	5	2	0
0.35	40.8 ± 3.2	5	3[a]	3[a]	5	1	0
0.20	38.5 ± 2.8	5	1	1	5	3	0
0.10	39.4 ± 2.3	5	0	0	5	4	0

[a] Significant difference from normal population; $P < 0.01$.

Table VI

Rotopad and Spasm tests following exposure to Halothane

Parameter	Body Weight (Mean ± St.Dev.)	Rotopad tests: Total Animals	Rotopad tests: Total Affected	Rotopad tests: Fell	Spasm tests: Total Animals	Spasm tests: Induced spasm	Spasm tests: Direct spasm
Total population: males and females							
	32.4 ± 4.3	531	28	6	469	140	0
Halothane exposure							
1%	39.4 ± 2.2	5	0	0	5[a]	5[a]	0
2%	39.0 ± 2.2	5	2[a]	2[a]	5	5[a]	0
4%	38.4 ± 1.5	5	5[a]	5[a]	5	5[a]	0

[a] Significant difference from normal population; $P < 0.01$.

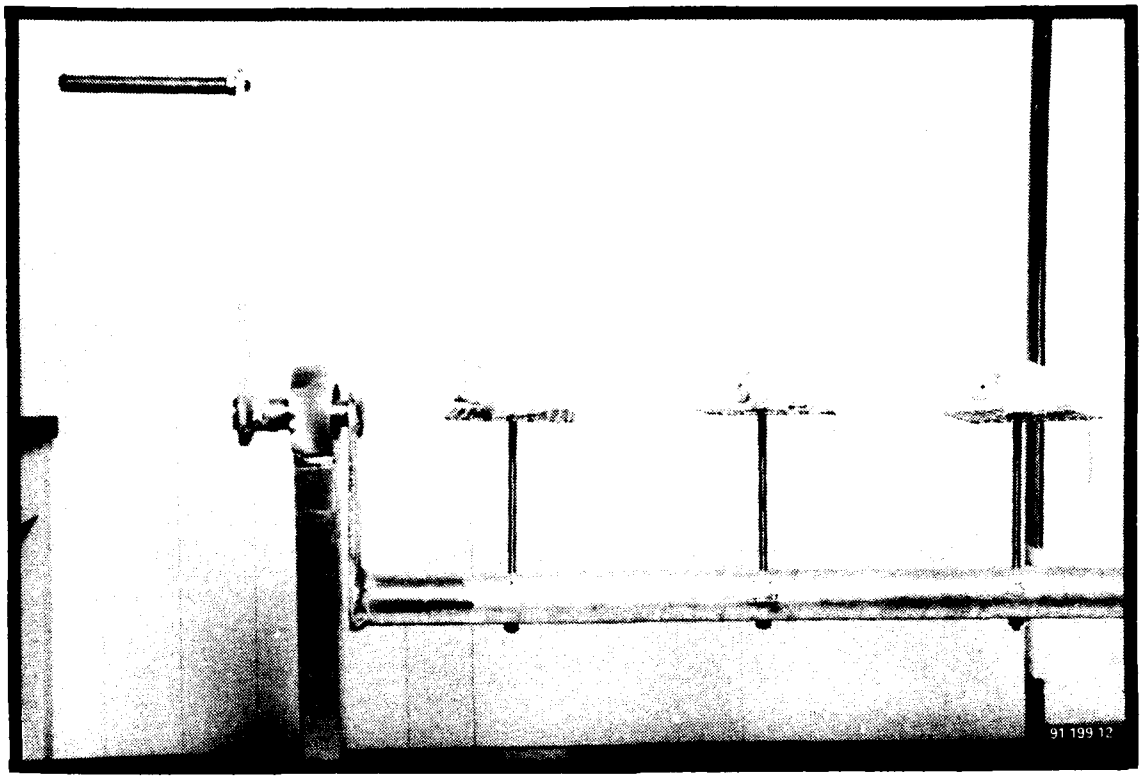

Figure 1

THE ROTOPAD APPARATUS CONSISTS OF A ROD SUSPENDED FROM A BEARING AT EACH END AND CONNECTED TO A TURNING HANDLE. WIRE MESH PADS, 12 X 12 CM, ARE MOUNTED ON 6 MM RODS AND FIXED TO THE ROTOROD SO THAT THE MICE DO NOT NORMALLY MOVE BETWEEN THE PADS. THE PADS ARE MOUNTED AT THE AXIS OF ROTATION TO MINIMIZE LATERAL MOVEMENT OF THE PADS DURING INVERSION. AT THE START OF THE TEST, THE MICE ARE PLACED ON THE TOP OF THE PADS.

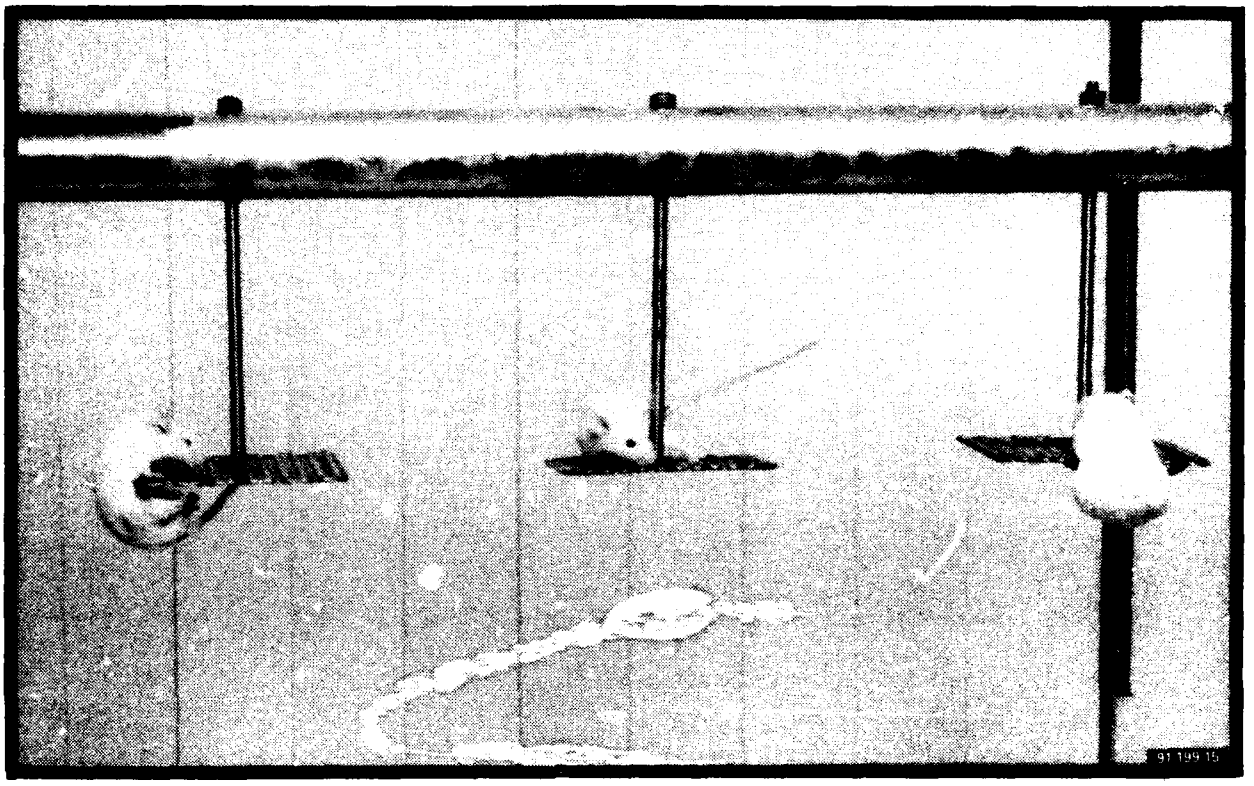

Figure 2

AFTER ROTATION, UNAFFECTED MICE CLIMB, INSTINCTIVELY, TO THE TOP SURFACE OF THE PADS.

A B C

Figure 3

TYPICAL SPASM RESPONSE IN THE CD-1 MOUSE. THE SPLAYED BRACHIAL AND DIGIT POSITION, ARCHED NECK AND GAPING MOUTH (A & B) INDICATE A TONIC CONVULSION. THERE IS GENERAL TONIC SPASM OF THE SKELETAL MUSCLES. THE CONVULSIONS CEASE WHEN THE ANIMAL IS BROUGHT IN CONTACT WITH A SURFACE. AN UNAFFECTED MOUSE IS SHOWN IN C FOR COMPARISON.

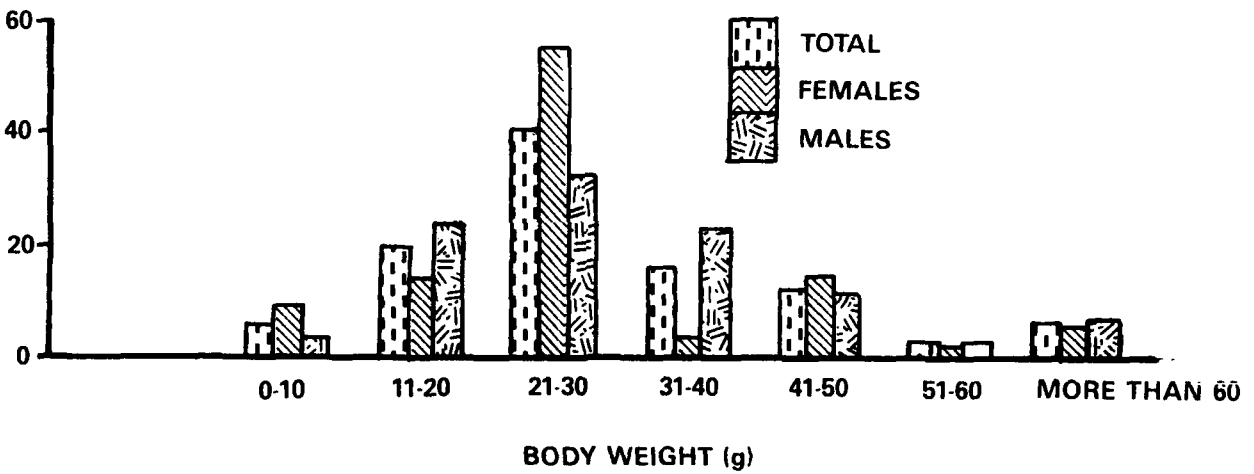

Figure 4

THE DISTRIBUTION OF TIMES REQUIRED FOR NORMAL MICE TO COMPLETE THE TEST SHOWS A MODE BETWEEN 20 AND 30 SEC IN BOTH MALES AND FEMALES. THE TEST WAS COMPLETED IN <60 SEC BY 94.7% OF THE MICE. THE FEMALES SHOWED A HIGH PROPORTION OF ANIMALS COMPLETING THE TEST BETWEEN 40 AND 50 SEC, AN UNEXPLAINED RESULT THAT APPARENTLY DOES NOT AFFECT THE FINAL OUTCOME.

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A simple test to indicate impaired motor function in mice is described. The test can be completed within 2 min using naive mice. Training of the animals is not a requisite. In normal mice, 94.7% of the population pass the test. Impaired motor function (Significant $P < 0.01$) is indicated when 2 of 5 animals fail the test. The effects of sodium pentobarbital, diazepam and chlorpromazine were studied to validate the procedure. All three drugs produced impaired function. Halothane anaesthesia (by inhalation exposure) was also tested but the recovery of the mice was too fast for reliable estimates of impaired motor function to be obtained.

A second test is described in which the tonic convulsions that occur spontaneously in CD-1 mice when they are suspended by the tail are used as a measure of seizure activity. Enhancement and suppression of the convulsions can be demonstrated. Chlorpromazine, halothane, diazepam and sodium pentobarbital all suppressed the spasm, the latter two at doses much below those required for effect in the rotopad test.

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